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BACTERIAL ACTIVITIES IN THE SUBAQUATIC SOILS OF LAKE ERIE

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During the summers of 1939 and 1940 a bacteriological study was made of the bottom soils of Lake Erie and its tributaries. This work was done for the Ohio Division of Conservation of which Dr. T. H. Langlois was director of research. Numerous samples were taken in different regions in order to study the role of micro-organisms in various physico-chemical phenomena. The area studied was divided into nine regions as follows: (I) Terwilliger's Pond which is a deep inlet directly connected with Hatchery Bay; (2) Hatchery Bay which connects Terwilliger's Pond with the lake proper; (3) Lake Erie proper which extends between the various islands; (4) Put-in-Bay Harbor which connects the lake proper with Squaw Harbor; (5) Squaw Harbor which is a shallow inlet of Put-in-Bay Harbor; (6) Pelee Island area extending around Pelee Island; (7) Portage River area; (8) Little Portage River area; (9) Haunck's Pond which is located in Middle Bass Island and is not directly connected with the lake unless wave action is quite great. All this region is in western Lake Erie within easy working distance from Put-in-Bay, Ohio. Particularly significant parts of the investigations dealt with the nitrogen transformations and the destruction of plant and animal materials, which have a direct bearing upon the quantity and quality of available food, and hence upon the fisheries industry.

METHODS AND TECHNICS

The samples were collected with dredges and placed in sterile wide-mouthed glass bottles. The samples were carefully removed from the center of the dredge core in order to reduce contamination to a minimum. Sterile wooden paddles were used in transferring samples to and from the bottles. Samples were tested immediately after being collected or were kept as cool as possible until examined. All technics employed were those recognized as standard in the bacteriological field. The agar plates were prepared by placing a gram of the soil in proper quantities of sterile distilled water to insure countable numbers of colonies on the agar plates. All inoculated media were incubated in electrically controlled incubators which operated at 20° C., 37° C. and 55° C. Agar plates were prepared in order to get an approximation of the relative numbers of organisms at various stations as revealed by the twenty-four and forty-eight hour incubation periods under aerobic and anaerobic conditions for each of the three temperatures mentioned (See Table 3).

In the study of the decomposition of mayflies a series of 500 cc. Erlenmeyer flasks was sterilized and inoculated with equal quantities of lake mud, lake water and adult mayflies as shown in Table 4. In samples AI, BI and CI the twenty mayflies were placed below the layer of mud and the flasks incubated at the temperatures designated. In samples A2, B2 and C2 twenty similar mayflies were placed in each of the flasks but were allowed to float upon the surface of the water, and incubated at the temperatures designated. The results are shown in Table 4. The optimum temperature for the complete as well as the most rapid decomposition seems to be 37° C., or slightly below (as in the case of sample CI). When a temperature below 37° C. was satisfactory for mayfly decomposition the covering of the mayflies with a slight coat of mud seemed to expedite the process. However, when buried in the mud too deeply the mayflies became filled with gas and "popped" out of the mud before actual decomposition was started.

In the study of the decomposition of green algae a series of sterile test tubes was inoculated with similar quantities of algae, lake mud and lake water. In each tube the strand of algae was imbedded in the mud and the remainder of the tube filled with lake water. The results of the decomposition in the mud and in the water above the mud are shown in Table 5. As with the mayflies, the optimum temperature for complete decomposition of the algae was 37° C. although complete decomposition also took place in a much longer time at a lower temperature. A slight degree of decomposition also took place at 55° C. in 4 days but the amount of destruction never advanced beyond this point within 64 days when the experiments were concluded.

In order to measure the amounts of gas produced during the decomposition process, equal quantities of mayflies, green algae as well as *Ceratophyllum* (hornwort) were placed in different sterile half-gallon fruit jars. In each jar a measured quantity of lake mud was placed in the bottom of the jar. The mayflies, algae or *Ceratophyllum* was then placed upon the mud. An inverted funnel with an inverted test tube on its stem were then placed over the inoculum. The jar, funnel and test tube were then completely filled with lake water. Incubations were made at 37° C. and 20° C. Controls without plant or animal materials were incubated under the same conditions to eliminate the possibility of having the gas production come from the mud rather than the inoculum. The jars were lightly covered with paper to reduce contaminations. The gas produced by the decomposition of the materials under the funnels was collected in the inverted test tubes as the water was displaced from them. The results are shown in Table 6.

MEDIA AND TESTS

In addition to routine, standard media the following were used for specific purposes. The demonstration of organisms capable of reducing urea to ammonia was made in Kappen's urea broth which was alkalinized with ammonium carbonate. The test for ammonia was made with Nessler's reagent. The detection of organisms capable of oxidizing ammonia to nitrites was made in Winogradsky's ammonium sulphate medium. The test for nitrites was made with the sulphanilic acid-alpha naphthylamine reagent. The demonstration of organisms capable of oxidizing nitrites to nitrates was made in sodium nitrite medium. The test for nitrates was made with diphenylamine reagent. When the test for nitrite was negative, one cubic centimeter of the culture was placed in a clean sterile tube and 2 drops of diphenylamine reagent and 2 drops of concentrated sulphuric acid were added. A blue color indicated nitrates (if the nitrite test was negative). To destroy the nitrites which interfered with the test, the culture to which sulphanilic-acetic acid was added, was kept in a water bath (boiling) for 20 minutes. Tests were made after heating to determine if nitrites had been eliminated. Tests for nitrates were then made. The successive reduction of nitrates to nitrites, to ammonia, and to free nitrogen was demonstrated in nitrate broth in test tubes in which there was an inverted smaller tube to collect the gas. The formation of foam on the surface of the medium, or of gas in the inverted tube, or both, showed the presence of free nitrogen. The detection of organisms (*Azotobacter sp.*) capable of fixing free nitrogen nonsymbiotically was done in Ashby's mannitol phosphate solution. The organisms appeared in a heavy, slimy pellicle after incubation under optimum conditions.

The test for cellulose-digesting organisms was made in McBeth's cellulose ammonium sulphate solution. Strips of sterile filter paper were placed in the tubed medium. After inoculation and incubation for the required period of time the following were noted: the time of paper discoloration; the evolution of gas; the odor; the time at which the paper began to decompose; when decomposition was complete (at which time Gram's stain was made). Eckford's method was used in determining the presence of organisms capable of hydrolyzing starch. Soluble starch (0.2%) was added to broth, and after proper incubation the medium was tested for acid production with an indicator of the proper pH-range. A drop of the inoculated medium was also placed in a depression of a porcelain plate and a drop of dilute iodine solution added. The reactions were read as follows: if blue, no hydrolysis; if reddish-brown, partial hydrolysis with the production of erythrodextrin; if clear, complete hydrolysis with the production of dextrin or perhaps glucose. Tests for sugars were made with Fehling's reagent. ZoBell and Feltham's technic for the determination of hydrogen sulphide was used. Ordinary broth was inoculated and incubated at the optimum temperature. A test strip of sterile filter paper soaked in a saturated solution of lead acetate was placed between the cotton plug and the mouth of the tube during incubation. Blackening of the test strip signified the presence of organisms capable of producing hydrogen sulphide. Indole-producing organisms were demonstrated by inoculating a solution of tryptone (0.1%). The Gnezda oxalic acid test showed the presence of indole. Strips of filter paper were immersed in a warm saturated solution of oxalic acid and then dried. A strip of the paper was inserted into the culture tube under aseptic conditions, and bent at such an angle that it pressed against the tube and remained near the mouth. The oxalic acid crystals on the paper turned pink if indole was formed. The presence of lactose-fermenting organisms was shown by inoculating lactose broth in Smith's fermentation tubes. The amount of gas was determined by means of a gasometer.

CONCLUSIONS

The data collected in connection with nitrogen transformations suggest the following conclusions:

(A) A reduction of urea to ammonia (in Kappen's urea broth) was observed in every sample in all stations tested. The reaction was recorded as strong in 42 stations (in 1½ to 3 days); moderate in 5 stations; and slight in 8 stations. It would appear that organisms capable of reducing urea to ammonia are quite generally distributed in the mud. (See Table 1).

(B) The oxidation of ammonia to nitrites (in Winogradsky's ammonium sulphate medium) was recorded as strong in 12 stations; moderate in 7 stations; slight in 27 stations; and negative in 9 stations. (See Table 1). These data would suggest that organisms capable of moderately or strongly oxidizing ammonia to nitrites, at least as revealed by laboratory conditions, are not as plentiful as the urea-reducing organisms.

(C) The oxidation of nitrites to nitrates (in sodium nitrite solution) was only slight in 15 stations and negative in 40 stations. (See Table 1). Even in stations where a slight test was positive, this occurred only after a minimum of 20 days. In many stations where a slight positive reaction occurred, a much longer time was required. It would appear that organisms capable of oxidizing nitrites to nitrates, at least as revealed by laboratory tests, are not as plentiful as the ammonia-oxidizing organisms. As a consequence, a minimum of nitrate formation for food purposes is to be expected. The real "bottle beck" in the production of nitrates from urea seems to be the result of a minimum of nitrite-oxidizing organisms, at least as revealed by laboratory data.

TABLE 1

Summary of Nitrogen Transformations

Station Number and Location		Reduction of Urea to NH_3 (Kappen's Urea Broth)	Oxidation of NH_3 to NO_2 (Winogradsky's Ammonium sulphate Solution)	Oxidation of NO_2 to NO_3 (Sodium Nitrite Solution)	Reduction of NO_3 to NO_2, NH_3 and N_2 (Nitrate Broth)	Azotobacter Organisms (Present in Days)
Terwilliger's Pond	9	Strong (2 days)	Slight (11 days)	None (45 days)	Strong (2 days)	6 days
	10	" " "	None (60 days)	" 60 "	Moderate (2 days	7 "
	11	" " "	Slight (50 days)	" " "	" " "	6 "
	11x	" " "	None (35 days)	" 45 "	Strong (3 days)	8 "
	11a	" " "	" " "	" " "	" " "	7 "
	12	" " "	Moderate (45 days)	Slight (30 days)	Moderate (2 days)	7 "
	13	" " "	Slight (13 days)	" 22 "	Strong (2 days)	6 "
	14	" " "	None (60 days)	None (60 days)	" " "	4 "
	15	" " "	Moderate (40 days)	Slight (45 days)	" " "	5 "
Hatchery Bay	16	Strong (2 days)	Slight (35 days)	Slight (50 days)	Moderate (3 days)	5 days
	17	Moderate (3 days)	None (60 days)	None (60 days)	" " "	3 "
	18	Strong (3 days)	Slight (65 days)	Slight (50 days)	" " "	3 "
	19	" " "	None (60 days)	None (60 days)	" " "	4 "
Lake Erie	36	Slight (3 days)	Slight (8 days)	None (50 days)	Moderate (2 days)	5 days
	37	" " "	" " "	" " "	" " "	5 "
	38	" " "	" " "	" " "	" " "	5 "
	39	Strong (3 days)	Moderate (5 days)	" " "	Strong (2 days)	6 "
	40	" " "	Slight (4 days)	" " "	" " "	6 "
	41	" " "	Moderate (4 days)	Slight (35 days)	" " "	6 "
	42	" " "	Slight (4 days)	None (50 days)	" " "	6 "
	43	" " "	" " "	" " "	" " "	6 "
Put-in-Bay Harbor	20	Strong (3 days)	Strong (33 days)	Slight (40 days)	Strong (2 days)	6 days
	21	" " "	" " "	None (60 days)	Moderate (2 days)	6 "
	22	" " "	" 31 "	Slight 45 days)	" " "	6 "
	23	" " "	Moderate (30 days)	" 42 "	" " "	5 "
	24	" " "	Slight (40 days)	None (60 days)	" " "	8 "
	25	" " "	Moderate (40 days)	" " "	" " "	6 "
	26	" " "	Slight (19 days)	Slight (20 days)	" " "	5 "
Squaw Harbor	55	Strong (1½ days)	None (35 days)	None (35 days)	Strong (1 day	6 days
	56	" " "	" " "	" " "	" " "	5 "
	57	" 3 "	" " "	" " "	" " "	6 "
Pelee Island	88	Strong (1½ days)	Strong (2 days)	None (6 days)*	Strong (2 days)	7 days
	88a	" " "	" " "	" " " *	" " "	7 "
	89	" " "	" " "	" " " *	" " "	7 "
	89a	" " "	" " "	" " " *	" " "	7 "
Portage River	44	Very strong (2 days)	Slight (17 days)	None (45 days)	Strong (2 days)	3 days
	45	" " " "	" " "	" " "	" " "	5 "
	48	" " " "	Strong (17 days)	Slight (35 days)	Moderate (2 days)	3 "
	49	" " " "	Slight (17 days)	None (50 days)	" " "	3 "
	50	" " " "	" " "	" " "	" " "	3 "
	51	" " " "	" " "	" " "	" " "	3 "
	52	Moderate (2 days)	Strong (17 days)	Slight (30 days)	" " "	3 "
	53	Very strong (2 days)	" 15 "	None (50 days)	" " "	3 "
	54	" " " "	Moderate (16 days)	Slight (18 days)	Strong (2 days)	3 "
Little Portage River	46	Very strong (2 days)	Strong (17 days)	Slight (50 days)	Strong (2 days)	3 days
	47	" " " "	" " "	None (50 days)	" " "	3 "
Haunck's Pond	27	Strong (3 days)	Slight (35 days)	Slight (42 days)	Moderate (2 days)	6 days
	28	Moderate (3 days)	" 41 "	None (60 days)	" " "	6 "
	29	" " "	" 14 "	" " "	" " "	6 "
	30	" " "	" 40 "	" " "	" " "	6 "
	31	Slight (3 days)	" 14 "	" " "	" " "	6 "
	32	" " "	" 14 "	" " "	" " "	6 "
	33	" " "	" 16 "	" " "	" " "	6 "
	34	" " "	" 14 "	" " "	" " "	6 "
	35	" " "	" 18 "	" " "	" " "	6 "

* Experiments discontinued at end of period designated.

(D) The reduction of nitrates to nitrites, to ammonia, to free-nitrogen (in nitrate broth) showed a strong production of ammonia (1–2 days) in 24 stations and a moderate production in 31 stations. A strong test for free nitrogen was present in 23 stations; moderate in 31 stations; and slight in 1 station. (See Table 1). These data suggest that organisms are prevalent which are capable of

TABLE 2

Summary of Cellulose Digestion, Starch Hydrolysis, Hydrogen Sulphide Production, Indole Production and Lactose Broth Fermentation

Location	Station Number	Cellulose Digestion	Starch Hydrolysis	Hydrogen Sulphide Production	Indole Production	Lactose Broth Percent of Gas	
						24 hours	48 hours
Terwilliger's Pond	9	None (30 days)	Complete (2 days)	Strong (1 day)	Strong (2 days)	60	90
	10	Complete (14 days)	" " "	" " "	Moderate (2 days)	35	70
	11	None (30 days)	" " "	Moderate (1 day)	Slight (2 days)	55	75
	11x	Complete (16 days)	Partial (35 days)*	Strong (2 days)	" 6 "	10	15
	11a	None (35 days)	" " " *	Slight (35 days)	" 8 "	5	10
	12	Complete (19 days)	Complete (2 days)	Strong (1 day)	Moderate (2 days)	70	95
	13	None (30 days	" " "	" " "	Slight (2 days)	60	90
	14	Slight (21 days)	" " "	" " "	" " "	55	80
	15	" " "	" " "	" " "	" " "	50	70
Hatchery Bay	16	None (35 days)	Complete (3 days)	Moderate (3 days)	Moderate (4 days)	45	75
	17	" " "	" 4 "	" " "	" " "	45	55
	18	" " "	" " "	" " "	" " "	60	90
	19	" " "	" 3 "	" " "	" " "	55	65
Lake Erie	36	None (27 days)	Complete (2 days)	Strong (3 days)	Slight (10 days)	60	100
	37	Complete (16 days)	" " "	" " "	" " "	55	90
	38	None (26 days)	" " "	" " "	" " "	55	95
	39	" 30 "	" " "	" 2 "	" 3 "	55	80
	40	Complete (23 days)	" " " *	" " "	" 10 "	60	80
	41	None (30 days)	" " "	" " "	" 10 "	80	90
	42	Complete (25 days)	" " " *	" " "	None (10 days)	80	90
	43	" 20 "	" " " *	" " "	Strong (7 days)	70	85
Put-in-Bay Harbor	20	Complete (45 days)	Complete (3 days)	Slight (3 days)	Slight (4 days)	80	90
	21	" 16 "	" " "	None (10 days)	" " "	80	90
	22	" " "	" " " *	Strong (3 days)	" " "	60	80
	23	" 18 "	" " " *	None (3 days)	" " "	60	80
	24	None (48 days)	" " " *	" " "	" 6 "	65	80
	25	" " "	" " " *	Strong (3 days)	" 4 "	75	80
	26	Complete (16 days)	" " " *	Slight (3 days)	" 6 "	80	90
Squaw Harbor	55	Complete (14 days)	Partial (30 days)*	Slight (23 days)	Slight (6 days)	40	70
	56	" 16 "	" " "	" " "	" " "	50	75
	57	" 17 "	" 25 " *	Strong (3 days)	" 7 "	50	75
Pelee Island	88	Not tested	Partial (5 days)*	Slight (6 days)	None (6 days)	0	10
	88a	" "	" " " *	Moderate (5 days)	" " "	0	10
	89	" "	" " " *	" " "	" " "	0	10
	89a	" "	" " " *	" " "	" " "	0	10
Portage River	44	Complete (23 days)	Complete (2 days)	Strong (2 days)	None (6 days)	45	70
	45	" 14 "	" " "	" " "	" 7 "	60	90
	48	" 18 "	" " "	" " "	" " "	55	85
	49	None (25 days)	" " "	" " "	" " "	60	95
	50	Complete (25 days)	" " "	" " "	" " "	50	80
	51	" 26 "	" " "	Moderate (2 days)	" " "	45	70
	52	" 25 "	" " "	Strong (2 days)	Slight (4 days)	50	85
	53	" 22 "	" " "	" " "	" " "	55	90
	54	" 20 "	" " "	" " "	Strong (2 days)	65	95
Little Portage River	46	None (28 days)	Complete (2 days)	Strong (2 days)	None (7 days)	60	90
	47	Complete (25 days)	" " "	" " "	" " "	60	90
Haunck's Pond	27	Complete (13 days)	Complete (3 days)*	Strong (3 days)	Slight (6 days)	10	60
	28	None (50 days)	" " " *	" " "	" " "	15	70
	29	" " "	" 4 " *	" " "	" " "	50	65
	30	Complete (13 days)	" " " *	" " "	" " "	5	50
	31	" 19 "	" " " *	" " "	" " "	5	50
	32	" 14 "	" " " *	" " "	" " "	0	60
	33	" 10 "	" " " *	" " "	" " "	20	50
	34	" 12 "	" " " *	" " "	" " "	0	50
	35	" 17 "	" " " *	" " "	" " "	70	100

* Butyric acid odor.

reducing nitrates rather extensively. Consequently, the balance between nitrate formation and nitrate reduction appears to be in favor of the latter.

(E) The presence of nonsymbiotic, free-nitrogen-fixing *Azotobacter* organisms was demonstrated in each of the 55 stations in 3 to 7 days when incubated at 20° C. in Ashby's mannitol phosphate solution (100 cc. in a 500 cc. Erlenmeyer flask). (See Table 1). The presence of these organisms does not prove their ability to fix free nitrogen in such soils as are found in the lake. Specific investigations will have to be made in this direction to clear up this point.

A study of Table I reveals consistent results for all stations in certain regions while considerable variations were noted in the different stations of other regions. For instance, in the Terwilliger Pond region, certain stations showed no nitrite production within 60 days while other stations in the same region showed some nitrite formation within 11 days. Similar phenomena are to be observed in other

TABLE 3

Average for All Stations in Each Area	Incubation Temperature	Average Number of Colonies on Agar Plates (Per Gram of Soil)			
		Aerobic		Anaerobic	
		24 Hour Incubation	48 Hour Incubation	24 Hour Incubation	48 Hour Incubation
Terwilliger's Pond	20° C.	45,000,000	65,000,000	37,000,000	51,000,000
Hatchery Bay	"	39,000,000	52,000,000	32,000,000	43,000,000
Lake Erie	"	33,000,000	48,000,000	39,000,000	50,000,000
Put-in-Bay Harbor	"	83,000,000	104,000,000	62,000,000	75,000,000
Portage River	"	40,000,000	59,000,000	33,000,000	44,000,000
Little Portage River	"	84,000,000	110,000,000	60,000,000	76,000,000
Haunck's Pond	"	60,000,000	85,000,000	51,000,000	65,000,000
Terwilliger's Pond	37° C.	54,000,000	74,000,000	45,000,000	60,000,000
Hatchery Bay	"	48,000,000	64,000,000	42,000,000	56,000,000
Lake Erie	"	45,000,000	65,000,000	44,000,000	60,000,000
Put-in-Bay Harbor	"	119,000,000	144,000,000	87,000,000	105,000,000
Portage River	"	53,000,000	73,000,000	46,000,000	61,000,000
Little Portage River	"	115,000,000	150,000,000	80,000,000	99,000,000
Haunck's Pond	"	43,000,000	64,000,000	30,000,000	37,000,000
Terwilliger's Pond	55° C.	39,000,000	50,000,000	26,000,000	38,000,000
Hatchery Bay	"	33,000,000	42,000,000	30,000,000	40,000,000
Lake Erie	"	25,000,000	37,000,000	28,000,000	38,000,000
Put-in-Bay Harbor	"	65,000,000	81,000,000	62,000,000	74,000,000
Portage River	"	48,000,000	61,000,000	46,000,000	60,000,000
Little Portage River	"	97,000,000	122,000,000	75,000,000	97,000,000
Haunck's Pond	"	Not determined	Not determined	Not determined	Not determined

regions. This may mean that the number of organisms necessary to oxidize ammonia to nitrites was too small to bring about the reaction; that the organisms were present but were prevented from acting normally; or that they were absent entirely.

The laboratory data suggested that there were no cellulose-digesting organisms in certain regions, while in other regions certain stations only showed them to be present. (See Table 2). When present, the cellulose was frequently completely digested in about two weeks. The presence of these organisms was noted in 33 stations while they were absent in 19 stations. The cellulose-digesting organisms initiate the destruction of plant materials which can be utilized by living organisms.

The organisms capable of hydrolyzing starch were present in all stations. In 46 stations there was complete hydrolysis while in 9 stations there was partial hydrolysis. (See Table 2). Partial hydrolysis was found in all stations of the Squaw Harbor and Pelee Island regions, and in 2 stations of Terwilliger's Pond. Evidently the organisms capable of changing starch so as to be usable by other living organisms are quite prevalent. In 25 stations the hydrolysis was attended by a butyric acid odor. (See Table 2).

The production of hydrogen sulphide varied from strong production in 1 day to none in 10 days. (See Table 2). Even in a particular region certain stations revealed strong production while nearby stations showed none. The production

TABLE 4

DECOMPOSITION OF MAYFLIES

SAMPLE NUMBER	TEMPERATURE	DECOMPOSITION	
		Started	Completed*
AI	55° C.	Slight (22 days)	Complete (60 days)
A2	55° C.	Slight (15 days)	Moderate (60 days)
BI	37° C.	Slight (5 days)	Complete (29 days)
B2	37° C.	Slight (7 days)	Complete (37 days)
CI	20° C.	Slight (7 days)	Complete (29 days)
C2	20° C.	Very slight (14 days)	Slight (60 days)

* Completed or when experiment was terminated.

In the above samples, mayflies were placed below the mud in sample numbers A1, B1, and C1, while the mayflies were placed upon the surface of the water in sample numbers A2, B2, and C2.

TABLE 5

DECOMPOSITION OF GREEN ALGAE

SAMPLE NUMBER	TEMPERATURE	DECOMPOSITION	
		Started	Completed*
D3	55° C.	Slight (4 days)	Slight (64 days)
D4	55° C.	Slight (4 days)	Slight (64 days)
E3	37° C.	Slight (4 days)	Complete (20 days)
E4	37° C.	Slight (4 days)	Complete (21 days)
F3	20° C.	Slight (29 days)	Complete (45 days)
F4	20° C.	Slight (35 days)	Complete (60 days)

* Complete, or when experiment was terminated.

of indole also varied in a similar manner. The production of gas in lactose broth was pronounced in a great majority of stations in 24 or 48 hours except in the Pelee Island region. (See Table 2). In the latter region a maximum of ten per cent of gas was produced in 48 hours. In a few stations in Haunck's Pond a slight quantity of gas was produced in 24 hours but a large quantity was formed after 48 hour incubation. These data do not necessarily mean pollution by intestinal organisms because they were not traced on differential media to prove their identity. Other types of aquatic organisms might also produce gas in lactose broth.

A study of data in Table 3 showing the average number of colonies (in millions per gram of soil) on agar plates reveals (1) that the averages for the stations in Lake Erie proper are usually below the average for other regions; (2) that the average for the Put-in-Bay Harbor region was high, particularly at 37° C. This is to be expected when the pollution problem is taken into consideration. Similar data were found in the Portage River area. Counts in the same station varied from time to time as repeated sampling revealed. The relative counts in all stations evidently were influenced by such factors as hydrogen ion concentrations, the quantity and quality of foods available, the amount of available oxygen, the presence of other micro-organisms which might live symbiotically or antagonistically with those being tested.

The experiments dealing with the decomposition of mayflies (Table 4) show that the optimum temperature for the complete as well as the most rapid decomposition was 37° C. (or slightly below as suggested by sample CI). When a temperature of 20° C. was satisfactory the mayflies apparently had to be covered with a coat of mud in order to expedite and complete the decomposition process. However, when covered too deeply the mayflies filled with gas and "popped" out of the mud before decomposition took place.

The decomposition of green algae (Table 5) also took place best at 37° C., although complete decomposition did occur at 20° C. but in a greater period of

TABLE 6

RATES OF DECOMPOSITION OF MAYFLIES, GREEN ALGAE AND CERATOPHYLLUM

(As Determined by Gas Production)

	37° C.		20° C.	
	Gas in Tube	Decomposition	Gas in Tube	Decomposition
I. Control	None (34 days)	....	None (34 days)	....
II. Ceratophyllum	Slight (5 days) Full (10 days)	Slight (31 days)	None (34 days)	None (34 days)
III. Green Algae	Slight (5 days) Full (10 days)	.... Complete (18 days)	None (34 days)	Slight (34 days)
IV. Mayflies	Much (5 days) Full (8 days)	.... Complete (27 days)	None (34 days)	None (34 days)

time. A slight degree of decomposition occurred at 55° C. in 4 days but the amount of destruction did not advance beyond that point within 64 days at which time the experiments were concluded.

The rate of decomposition of mayflies, algae and hornwort (*Ceratophyllum*) as shown by gas production (See Table 6) also revealed the optimum temperature to be 37° C. In fact, at 20° C. incubations, no gas was produced in 34 days by any of the three materials tested. Only the algae were slightly decomposed in 34 days when incubated at 20° C. However, at 37° C. the production of gas was extensive and the decomposition was complete for the algae in 18 days and for the mayflies in 27 days. The *Ceratophyllum* decomposed only slightly in 31 days at 37° C. which might suggest its resistance to destruction by the quantity and quality of the particular micro-organisms present.

The decomposition of the plant and animal materials, if the temperature and other factors are satisfactory, occurs rather rapidly thus making available their constituents for use by future organisms. It would appear that the organisms responsible for optimum decomposition of the materials tested are those of animal origin because their optimum temperature was 37° C. The aquatic types of micro-organisms whose optimum temperatures are expected to be below 37° C. probably play a minor role in this phenomenon. This is also true for the thermophilic micro-organisms whose optimum temperature is 55° C. The presence in rather large numbers of micro-organisms whose optimum temperature is 37° C. as revealed by laboratory tests of the soil samples, undoubtedly substantiates this conclusion.